California Bovine Trichomonosis Program Frequently Asked Questions

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TREATMENT

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TRICHOMONOSIS: THE DISEASE

1. What is the prevalence of trichomonosis infection in California cattle?

15.8% (9/57 herds) of California cow-calf herds were affected with trichomonosis in a 1990 study (BonDurant RH, et al; Prevalence of trichomoniasis among California beef herds; *J Am Vet Med Assoc* 1990; 196(10): 1590-1593). This study tested 729 bulls from 57 herds. Herd size, as judged by number of bulls, ranged from 1 to 210 bulls (median = 6 bulls per herd).

CDFA expects to garner a better understanding of current trichomonosis prevalence in California in the coming years thanks to updated regulations requiring all positive and negative trichomonosis tests be reported.

2. What are the economic costs of trichomonosis in California?

True economic costs of trichomonosis are difficult to quantify. One study out of UC Davis estimated that combinations of risk factors contributed to a loss of income up to 22% (Villarroel A, Carpenter TE, BonDurant RH; Development of a simulation model to evaluate the effect of vaccination against *Tritrichomonas foetus* on reproductive efficiency in beef herds; *Am J Vet Res* 2004; 65(6): 770-775). This study found shared grazing to be the most significant risk factor for a decrease in calving incidence attributable to *T. foetus* infection. Additional risk factors included bulls not being tested negative for *T. foetus* prior to breeding and herds containing a higher proportion of older bulls (bulls >3 years of age).

Another disease model predicted, "a reduction of 14 to 50% in annual calf crop, a prolonged breeding season, a reduction of 5 to 12% in the suckling / growing period, a reduction of 4 to 10% in pounds of marketable calf crop at weaning, a reduction of 4 to 10% in monetary return per calf born, and a substantial reduction of 5 to 35% in the return per cow confined with a fertile bull." (Rae DO. Impact of trichomoniasis on the cow-calf producer's profitability. *J Am Vet Med Assoc* 1989; 194 (6): 771-775).

Losses to cow-calf herds include cost of replacement bulls, loss of genetic potential due to culling, increased days-to-conception, and subsequently lighter weaning weights.

3. Is there threat to human health from *Tritrichomonas foetus*?

Tritrichomonas foetus infections have not been documented in humans, nor are there any known risks to humans for contracting this disease from cattle. *T. foetus* is a venereal disease of cattle; it has not been documented as a cause of venereal disease in other species. **This protozoa has not been found to cross mucosal barriers from genital organs into the blood or meat of infected animals**.

However, trichomonads have been discovered in other species. A trichomonad closely related to *T. foetus* was identified in a bronchoalveolar lavage sample from a patient with AIDS in association with Pneumocystis pneumonia. Additionally, there is a single report of a man being treated with subarachnoid (space around brain) anti-cancer drugs that were somehow contaminated with *T. foetus*; this patient subsequently died of protozoal myelitis. *T. foetus* has also been identified as a cause of diarrhea in cats. These findings have brought into question previously held beliefs that trichomonads are species specific. Research is active and ongoing to determine how closely trichomonads in different species are related and if any zoonotic potential exists.

4. How significant a risk are carrier cows for maintaining infection in a herd?

Cows are potential sources of new infections and maintaining the organism in a herd. Usually they only harbor the organism for a few heat cycles after infection or pregnancy loss. Some cows can carry the organism through the gestation period and well into the postpartum period. Skirrow found two of 40 infected cows from two herds that carried the infection through the entire gestation and for up to nine weeks postpartum (Skirrow SZ. 1987; Identification of Trichomonad-Carrier Cows; *J Am Vet Med Assoc*; 191:553-554).

Such a 'carrier cow' presents a problem when trying to control the disease and offers at least partial explanation for the persistence of infected animals when control measures have concentrated on eliminating positive bulls. True prevalence of carrier cows is unknown, but is thought to be less than one (1) percent. These cows may be clinically affected (abortion, infertility, pyometra) or there may be no apparent clinical signs.

TESTING

1. Official Identification is required for every bull tested: What forms of ID are official?

Unique official identification is required for each animal tested.

Examples of approved individual official identification include USDA metal silver brite tags; USDA approved electronic identification devices, and breed registry tattoos with corresponding paperwork.

Tags that only have a herd id number such as bangle ear tags, brands, and backtags are not considered official identification for the trichomonosis program.

2. Who can perform official trichomonosis testing?

Only California licensed, USDA accredited veterinarians who have completed training approved by CDFA in sampling and handling of specimens used in the diagnosis of trichomonosis are eligible to perform official trichomonosis testing in California.

3. What constitutes an official trichomonosis test?

A test for the detection of active infection with *Tritrichomonas foetus* from a specimen (sample taken from the preputial cavity of a bull, or the uterine contents of a bovine female) collected by or under the supervision of a trichomonosis approved veterinarian and conducted at a trichomonosis approved laboratory.

A negative test result from a bull is an official test only if the specimen is collected following ten (10) days without contact with sexually mature female cattle.

Official identification of the animal tested must be recorded and accompany the sample to the laboratory. Trichomonosis test results must be recorded on forms approved by the Department of Food and Agriculture for that purpose. Copies of all test results shall be sent to the Department of Food and Agriculture within 30 days of the test results. Positive tests must be reported within two (2) days of test result.

4. Is there a blood test for trichomonosis?

No, there is no commercially available serologic test for trichomonosis. Dr. Bob BonDurant's Trichomonosis Research Lab at the UC Davis School of Veterinary Medicine has been researching blood testing for diagnosing trichomonosis. The test they have been working with is called a "hemolytic" assay; this is actually an old fashioned Complement Fixing assay, in which bovine red blood cells from known negative cows are coated with *T. foetus* antigen, then exposed to titrated test sera or negative control sera. Following the addition of rabbit or guinea pig complement, the red cell suspension lyses if there is Complement Fixing antibody in the serum. If not, the RBC's just sink to the bottom, forming a pellet. No other labs are running this test, and Dr. BonDurant's lab is currently only running the test for research (when results may offer significant diagnostic information or research advancement). The CF test described above has shown some benefit in trying to determine exposure of a herd to trich, but results are meaningless if the herd has been vaccinated in the past 18 months or so. A Polymerase Chain Reaction (PCR) test using serum (for trichomonosis DNA) is unlikely to be of use, since it is believed this organism never gets across mucosal barriers into the blood.

5. How do we know if our vet is approved?

The easiest way to know if your veterinarian is approved for sampling and if their clinic is approved to read collected samples is to ask your veterinarian. You may also notice certificate(s) posted in their office showing approval for sampling and/or reading samples. Approval can be confirmed by Contacting your nearest Animal Health Branch District Office.

6. Can non-veterinarians perform official trichomonosis testing?

Official trichomonosis testing must be done under the supervision of a veterinarian approved for sampling.

Testing performed without the direct or indirect supervision of an approved trichomonosis veterinarian will not be accepted for regulatory requirements, including sale and interstate movement. The terms 'direct supervision' and 'indirect supervision' in this context only apply to registered veterinary technicians and unregistered assistants employed by the approved trichomonosis veterinarian. CDFA expects these technicians and assistants have been instructed on proper sample handling and collection by the trichomonosis approved veterinarian; instruction should include watching the CDFA DVD, "Bovine Trichomonosis: Bull Sampling and Diagnosis". The veterinarian must have an existing valid Veterinarian-Client-Patient Relationship (VCPR) with the herd owner and animals being tested if their technician or assistant will be collecting samples under indirect supervision.

Herd owners may test their own bulls for herd health purposes, but test results will not be considered official for regulatory requirements, such as for sale, interstate movement, or to release affected or exposed herds from quarantine.

Samples for trichomonosis must be read by an approved laboratory to be considered official tests.

Any positive trichomonosis test result(s), regardless of source, must be reported to CDFA within two days, and CDFA will investigate the source of the positive test(s) and possible exposure to neighboring herds. This requirement to report positive test results includes testing done by herd owners and lay testers, pursuant to Section 9101 of the California Food and Agricultural Code.

7. What if a certified semen collector diagnoses trichomonosis?

Any positive trichomonosis test result(s), regardless of source, must be reported to CDFA within two days, and CDFA will investigate the source of the positive test(s) and possible exposure to neighboring herds.

Testing is official for sale and interstate movement only if they are working under the supervision of a veterinarian approved for sampling. That veterinarian must have a valid Veterinarian-Client-Patient Relationship (VCPR) with the herd owner and animals being tested.

8. Is testing only required for saleyard bulls?

Trichomonosis testing in California is required for all bulls 18 months of age and older sold through a public saleyard. If bulls do not have a negative trich test on arrival at the saleyard, they will be marked to go directly to slaughter or through an approved feeding channel to slaughter.

Green tags placed on bulls at saleyards are a visual indicator that the bull is only to be sold for slaughter. Brand inspectors or livestock inspectors will place a green tag on any bull that does not have a negative trichomonosis test and is 18 months of age and older.
Lack of a green-tag does not definitely indicate the bull has a negative trichomonosis test, as each saleyard may have alternative ways to identify tested versus untested bulls.

California regulations do not have specific requirements for bulls sold via private treaty; however, we recommend buyers require a negative trichomonosis test and a breeding soundness examination (semen test) on any bulls being purchased for breeding.

Trich testing is also required for bulls 18 months and over entering California. This test must be done within 60 days of entry. Exceptions to the test requirement are: bulls going direct to slaughter, bulls on exhibition that have no contact with female cattle, and bulls entering California to be used solely for artificial insemination using protocols that meet Certified Semen Services (CSS) standards.

Bulls entering California on a Pasture-to-Pasture permit require a negative trich test within 12 months.

9. Aging cattle: how do I know if my bull is 18 months old or older?

Cattle with eruption of one or more central adult incisors are considered to be 18-24 months of age. If all deciduous (baby) teeth are still present, the animal is considered to be 15-18 months of age.
For more detailed information, please refer to the document: Using Teeth to Age Cattle, available under 'For Public Sale Yards' on CDFA's trichomonosis web page.
Information and photos in this document were taken from the USDA-FSIS webpage:
http://www.fsis.usda.gov/ofo/tsc/bse_information.htm

10. What does a 'green tag' mean on a saleyard bull?

Green tags placed on bulls at saleyards are a visual indicator that the bull is only to be sold for slaughter. Brand inspectors, working with livestock inspectors, will place a green tag on any bull that does not have a negative trichomonosis test and is 18 months of age and older.

11. Can bulls be tested for trichomonosis at the sale yard?

Yes; however, **the test must be performed prior to sale, with full and informed consent of the seller**. If the test is positive for *T. foetus*, the source herd of the bull will be investigated, as will any neighboring herds. The positive bull, and any subsequent bulls diagnosed as positive during the investigation, will be quarantined until they go to slaughter.

The buyer, seller and saleyard are expected to negotiate who pays for saleyard trich testing.

Bulls tested at the saleyard should not go through a sale or leave the saleyard premises until a final negative trichomonosis test result has been reported to the approved testing veterinarian by the approved laboratory reading the sample(s).

12. Can bulls be tested for trich AFTER they have gone through sale ring at a saleyard?

No. California's trich regulations state that bulls must have a negative test within 60 days PRIOR TO SALE or be sold only to slaughter. If a buyer wants to purchase a bull that does not have a negative trich test, the seller should be contacted and the bull should be pulled from the sale, held at the yard until a negative test result is obtained, and then put through the ring. Alternatively, the seller could take the bull home for official testing, and then return the bull to the saleyard to be sold once the bull tests negative for trich.

13. Are there testing requirements for private treaty sales?

No. California does not have a trich test requirement on bulls sold via private treaty. This requirement was purposely left of out the regulations because the cattle industry and CDFA felt that uniform enforcement would be difficult to impossible. Additionally, it was felt that there would be more accountability in a private treaty sale to sell a bull free from trich infection because of the face-to-face interaction between buyer and seller.

CDFA strongly recommends that all individuals purchasing bulls via private treaty for breeding require bulls to be tested negative for trich at least one time before sale.

14. Why must bulls have no contact with sexually mature female cattle for the 10 days just prior to testing?

This down time allows protozoal numbers in the epithelial crypts of the prepuce and penis time to multiply to detectable levels. Bulls may test negative, even if they are actually infected with *Tritrichomonas foetus* (false negative) without this period of sexual rest.

15. What is the time frame between the three negative tests required for bulls from affected herds?

Each test must be conducted at least seven (7), but not more than 28 days apart. Testing on bulls must be conducted following 10 days without contact with sexually mature female cattle.

16. What if I had bulls test positive for trich on pre-breeding herd testing, but have already turned out remaining bulls for breeding, making them unavailable for the three (3) required negative tests?

Generally, if bulls in an affected herd (any herd with one or more bulls test positive for *T. foetus*) have been turned out for the breeding season prior to being tested negative three (3) times, bulls may remain quarantined to the premises until they are gathered and tested following the breeding season. Once they are done with the current breeding season, all herd bulls in an affected herd require three (3) negative tests conducted at least seven (7), but not more than 28 days apart. Bulls must have no contact with mature female cattle for at least 10 days prior to each test.

There may be circumstances where districts may require bulls from affected herds to be gathered and tested before the breeding season is finished. CDFA District office personnel will work with owners to set up a plan for investigation and testing of all affected and exposed herds.

District animal health personnel will begin an epidemiology investigation of the affected herd and contact exposed neighboring herd owners following the first positive test from a herd; contacts with neighbors may be initiated before all testing on the affected herd is complete. If these neighbors share grazing allotments, they may opt to pull their bulls out of shared grazing areas and complete the one (1) required test on all herd bulls prior to completion of affected herd testing. If bulls from exposed herds are tested in this manner, they should not be returned to grazing allotments shared with bulls from the affected herd.

17. Why are three (3) negative tests required for affected herds?

Studies of known "positive" bulls have shown that the culture method will miss about 10-20% of infected bulls if we only test them once; testing the herd (all the bulls in the herd) once gives us an 80-90% chance of finding the disease if it's there. If no infected bulls are found on the basis of a single culture of all bulls, then we can be 80-90% sure that the herd is "clean" (free of trichomonosis).

At the second test, we are trying to find infected bulls that were not detected on the first test; this means we are looking for bulls called negative on the first test when they were infected (false negatives). After a second test we can be 80-90% + 8-9% more certain we have found all infected bulls, so 88-99% sure the herd is not affected with trich. A third consecutive test of all herd bulls allows us to be more than 99% sure that the entire bull herd is free of disease.

18. Who pays for required testing of affected or exposed herd bulls?

The bull owner is expected to pay for all required testing.

19. Why isn't it the responsibility of the owner of the affected herd to pay for exposed herd testing?

We do not know that the herd diagnosed with trichomonosis is the source of infection; therefore, we cannot make testing all neighboring herds the responsibility of that owner. The original source of infection may be one of the neighboring herds or a different source entirely.

RESULTS / DIAGNOSIS

1. What percentage of tests has a false positive result?

False positives can occasionally occur; CAHFS lab data suggests the false positive rate is less than five percent (<5%) of all positive cultures read by the lab. When false positives do occur, it is most likely due to the presence of a fecal trichomonad. Fecal trichs are routinely detected in young, group-raised virgin bulls as well as in mature breeding bulls. If a virgin bull cultures positive, it is more likely to be a NTfT (non *T. foetus* trichomonad or fecal trich).

Polymerase Chain Reaction (PCR) can confirm culture results. PCR confirmation is recommended, but not required, for herds diagnosed with trichomonosis for the first time or if positive culture results are obtained from virgin bull samples. Specific PCR primers exist to differentiate *Tritrichomonas foetus* from fecal trichomonads. PCR testing can be done through CAHFS laboratories.

2. How many Trich species can be detected in cattle?

Three genera, with multiple species, make up the majority of trichomonads found in cattle: Tritrichomonas, Tetratrichomonas, and Pentatrichomonas. Within these categories, only one species of Tritrichomonas (*Tritrichomonas foetus*), many species of Tetratrichomonads, and one species of Pentatrichomonas (*Pentatrichomonas hominis*) are routinely detected in cattle. *Tritrichomonas foetus* is the only one of these protozoa that causes trichomonosis.

3. Who is required to report the positive test, owner or veterinarian?

The veterinarian approved for trichomonosis sampling that collects the samples, is expected and required to report all positive and negative test results to CDFA. Positive tests must be reported within two days of the final lab reading date, and negative tests must be reported within 30 days.

Veterinarians submitting samples to CAHFS laboratories for reading should submit the top three copies of the Trichomonosis Test Report Form (AHB 76-199) to the lab with samples. CAHFS will report results directly to the appropriate Animal Health Branch District Office and return the owner and veterinarian copies to the submitting veterinarian.

However, according to the California Food and Agriculture Code of Regulations, section 9101, any licensed veterinarian, any person operating a diagnostic laboratory, or any person who has been informed, recognizes or should recognize, by virtue of education, experience, or occupation, that any animal or animal product is or may be affected by, has been exposed to, or may be transmitting or carrying any condition specified in the "List of Reportable Conditions for Animals and Animal Products," shall report to the department all known information required by the department within the time specified in the "List of Reportable Conditions for Animals and Animal Products."

4. My bull tested positive on culture, but I do not think he is truly infected – what can I do?

False positive results will occasionally occur. Fecal trichomonads will grow in *Tritrichomonas foetus* culture media and can be mistaken for *T. foetus*. This situation is most likely when young, group-raised virgin bulls test positive on culture for *T. foetus*.

The animal owner or testing veterinarian can request culture results be confirmed by Polymerase Chain Reaction (PCR). Specific PCR primers exist to differentiate *Tritrichomonas foetus* from fecal trichomonads. PCR testing can be done through CAHFS laboratories. If the sample is negative for *T. foetus* and positive for other trichomonads, the bull will be released from quarantine.

TREATMENT

1. Why can't we use antibiotics like they are using in humans to treat this disease in bulls?

No approved, effective treatments exist to treat bulls or cows for trichomonosis at this time.

CONTROL

1. My herd was diagnosed with trichomonosis: What happens now?

- The testing veterinarian and/or laboratory that read the test will report the positive result to their local Animal Health Branch District Office within 2 days.

- District personnel will quarantine infected animals and herdmate bulls. Infected bulls are permanently quarantined until they go to slaughter. Herdmate bulls require three (3) consecutive negative trichomonosis tests to be released from quarantine.

- District personnel will investigate the infected herd, including gathering information from the owner about herd management and risk factors for trich. Additionally, personnel will collect information on all locations herd bulls have been during the past 12 months and any known or possible contacts with neighboring herds that may have occurred in the past 12 months.

- Herdmate bulls must have three (3) consecutive negative trichomonosis tests conducted by an approved private veterinarian at least seven (7) days, but not more than 28 days apart. All tests must be performed following 10 days sexual rest (no contact with sexually mature female cattle).

- If an approved trichomonosis veterinarian did the first test, the test is within the specified time frame (three tests at least seven but not more than 28 days apart), and the test included all herdmate breeding bulls, it will count as the first of the three required tests.

- Bulls diagnosed as infected with trichomonosis at any of the three tests will be permanently quarantined until they are slaughtered; remaining herd bulls will be released from quarantine once they test negative on three consecutive tests.

- While the investigation is taking place, until appropriate testing has been performed, and the Department has released the herd, all herdmate breeding bulls within the herd will be held on the premises where the infection was diagnosed.

- Movement off premises may be allowed with written permission from CDFA. If the owner of the herd is non-compliant with testing requirements, the whole herd may be restricted from movement off the premises where infection was diagnosed.

- Written confirmation of slaughter is required when infected cattle are slaughtered.

2. My herd was diagnosed with trichomonosis: How do I get trich out of my herd and keep it out?

- Test all bulls three times and send all infected bulls to slaughter.

- Heed the adage, “good fences make good neighbors” – keep fences in good repair to keep neighboring bulls out of your herd. Commingled grazing or fence-line contact with other herds is a documented risk factor for trichomonosis infection.

- Trich test all herd bulls annually prior to breeding, at the time of annual fertility exams.

- Maintain a defined breeding season – this will help identify reproductive problems more readily and will produce a more uniform calf crop.

- Pregnancy test all cows and heifers after the breeding season and cull open females.

- Segregating cows based on gestation length at the time of trichomonosis diagnosis may be considered:
    - Cows pregnant five or more months have the lowest risk of being infected.
    - Cows pregnant less than five months should be pastured separately and observed closely. Cull any of these cows that abort.
    - Open cows with or without pyometras or other uterine abnormalities should be culled.

- Maintain a closed herd.

- Maintain a young (<4 years old) bull battery – cull older bulls.
- If unable to maintain a closed herd, purchase only virgin, yearling bulls.
- Do not share or lease bulls.
- Maintain separate breeding groups. This way, if one bull is infected with trichomonosis, he will not spread it through the entire herd. Breeding groups must have no contact with each other for this to be an effective control strategy.
- Use artificial insemination. Artificial insemination is considered the classic method for controlling venereal diseases of cattle. However, this is often impractical in range cattle operations due to lack of facilities, expertise or management practices.
- Vaccinate cows to decrease duration and severity of infection. Vaccines, when used according to label directions, show effectiveness in the female, but not in the bull.

3. I lease bulls: After a leased bull was returned, he tested positive for trich; do I need to test all my bulls?

It depends.
If the leased bull tested negative on an official trichomonosis test just prior to leaving your premises, did not have any contact with other breeding age cattle following that test until arriving at the premises he was leased to, and when he arrived back at your premises he was quarantined away from all bulls and cows until the official positive test result was obtained, then testing of all other bulls you have available for lease should not be necessary.

CDFA Animal Health Branch personnel investigate all positive trichomonosis test results. As part of this investigation, personnel will want to know about all locations positive bulls have been during the past 12 months and all potential contacts with other cattle during that 12 month period, including herdmates and neighboring herds.

Your Animal Health Branch District Office should be contacted if you have specific questions.

4. My neighbor's herd was diagnosed with trichomonosis: What happens now? Do I have to test my bulls?

- District AHB field personnel will conduct an epidemiologic investigation on any herds identified as being potential exposures to affected herds.
- Herdmate bulls in exposed herds will be quarantined to premises, and **must have one (1) negative trichomonosis test** conducted by an approved trichomonosis veterinarian to be released from quarantine.
- If any bulls in exposed herds test positive for trichomonosis, the herd will be treated as an affected herd.
- Movement off premises may be allowed with written permission from CDFA. If the owner of the herd is non-compliant with testing requirements, the whole herd may be restricted from movement off the premises where infection was diagnosed.

5. My neighbor's herd was diagnosed with trichomonosis: How do I keep trich out of my herd?

- Heed the adage, "good fences make good neighbors" – keep fences in good repair to keep neighboring bulls out of your herd. Commingled grazing or fence-line contact with other herds is a documented risk factor for trichomonosis infection.
- Maintain a closed herd.
- Trich test all herd bulls annually prior to breeding, at the time of annual fertility exams.
- Maintain a young (<4 years old) bull battery – cull older bulls.

- If unable to maintain a closed herd, purchase only virgin, yearling bulls.
- Do not share or lease bulls.
- Maintain separate breeding groups. This way, if one bull is infected with trichomonosis, he will not spread it through the entire herd. Breeding groups must have no contact with each other for this to be an effective control strategy.
- Maintain a defined breeding season – this will help identify reproductive problems more readily and will produce a more uniform calf crop.
- Pregnancy test all cows and heifers after the breeding season and cull open females.
- Use artificial insemination.

6. Is there a vaccine for trichomonosis? / What is the protocol for using the vaccine?

Yes, Fort Dodge makes a killed vaccine, labeled for cattle, that is shown to help decrease severity and duration of infection (helps clear infection in cows faster). Directions on the label read: Cattle, inject one 2mL dose subcutaneously under aseptic conditions. A second dose should be administered 2 to 4 weeks later. The last injection should precede the breeding season by 4 weeks. Revaccinate annually. Vaccination should be done 30 days prior to turning bulls in with cows.

Vaccination will not prevent transmission of and infection by *Tritrichomonas foetus*. Because the infection is transmitted during breeding, infection will occur. However, vaccination will limit the duration of infection and result in more pregnancies being carried to term. If your herd has been diagnosed with trich or if you are aware of significant risks for introduction of infection into your herd, vaccination may help decrease your immediate economic losses; however, testing and culling bulls in addition to culling any open cows are the most important steps to take in clearing the infection from your herd. Most abortions attributable to *T. foetus* occur around 60-70 days after breeding with infected bulls, but some abortions do occur later in gestation. Consideration should be given to segregating short-bred cows and re-evaluating their pregnancy status at a later time. **Vaccination alone, without implementation of other control measures, is of limited value.**

There is some suggestive evidence that properly vaccinated bulls can resist infection following experimental inoculation with *T. foetus* organisms; vaccination of bulls needs to be studied much more extensively and with a large number of bulls before any recommendations for widespread use can be made.

VIOLATIONS

1. What happens if green tags placed on bulls at saleyards are removed so they can be sold without restrictions?

CDFA has the authority to issue violations to anyone not following regulations. If anyone is seen removing green tags placed by Brand or Livestock Inspectors on bulls without a negative trich test, the CDFA District Veterinarian In Charge (VIC) in that area should be notified. The VIC, in consultation with CDFA Headquarters veterinarians, may decide to issue a violation to the offender. Fines can be up to $500.00 per violation per day. Each bull that had a tag removed would be considered a violation.

2. What if a saleyard is selling bulls with green tags to people who do not plan to slaughter these bulls?

CDFA has the authority to issue violations to anyone not following regulations. If saleyards are observed intentionally selling bulls with green tags for purposes other than slaughter, the CDFA District Veterinarian In Charge (VIC) in that area should be notified. The VIC, in consultation with CDFA Headquarters veterinarians, may decide to issue a violation to the offender. Fines can be up to $500.00 per violation per day. Each bull with a green tag that is sold for purposes other than slaughter could be considered a violation.

VETERINARIAN QUESTIONS

1. How do I become approved to collect samples for trichomonosis testing?

California licensed, USDA accredited veterinarians can request a training video to become approved for trichomonosis sample collection in California through their Animal Health Branch District Office. This DVD will be mailed to the requesting veterinarian to watch independently; it will arrive with a form that must be returned to the district office once the veterinarian has watched the training video. A certificate of training for sampling and handling will be mailed from AHB headquarters following receipt of this form at the district.

Once the form has been received by CDFA, you are approved for sampling and handling of trichomonosis samples. Approval is renewed every two years with CDFA at the same time Brucellosis contracts are renewed.

2. How can my clinic / laboratory become approved to read trichomonosis samples?

Clinics can become approved to read trichomonosis samples by sending someone from the clinic to a training session at a California Animal Health & Food Safety (CAHFS) Laboratory. This person may be a veterinarian or another clinic employee designated by the clinic / laboratory.

Multiple individuals from a clinic are welcome to attend training sessions; however, please inform CDFA in advance of the names and job functions (veterinarian, technician, RVT) of all people planning to attend so the lab can plan accordingly. Each CAHFS lab training session lasts about one hour, and is limited to no more than six (6) people.

The expectation with lab training is that the person trained at CAHFS will go back to the clinic and share knowledge gained with all individuals reading trichomonosis samples.

If the person attending this lab training subsequently leaves the clinic, then that clinic/lab is no longer approved to read trichomonosis samples. If this happens, please contact CDFA to schedule another person for lab training.

Scheduling can be done online at: http://et.cdfa.ca.gov/trich/selfreg/ . If veterinarians / clinics do not have web access, they can contact their Animal Health Branch District Office to complete the online scheduling form. CDFA will then contact the individual to confirm training date and location.

3. When and to where do I need to report test results?

All positive tests must be reported to your local Animal Health Branch District Office within two (2) days of the final read date.

All negative tests must be reported to your local Animal Health Branch District Office within 30 days of the final read date.

All test results should be reported on CDFA's Trichomonosis Test Report Form.
If a CAHFS laboratory reads the samples, they will report results to the Animal Health Branch.

4. PCR confirmation of a culture diagnosis was requested: how should results be reported?

Bull owners or their testing veterinarian may request PCR confirmation of a positive culture result. If confirmation by PCR has been requested, your Animal Health Branch District Office should be informed of the positive culture result, but the positive test result will not be investigated until the PCR result is reported. For the positive culture result to be reclassified as a negative official test, the PCR must be negative for *T. foetus* and positive for other trichomonads. CAHFS laboratories will report final test results directly to CDFA, so the testing veterinarian will not need to do so.

The laboratory will report PCR results as follows:

- If the bull is PCR positive, using primers specific for *T. foetus*, he is declared infected.
- If the bull is positive for trichomonads in general, but negative for *Tritrichomonas foetus*, the bull is reported as negative.
- If the PCR is negative for all trichomonads, it suggests that there was an insufficient amount of DNA present to be detected by the PCR assay or that the DNA was somehow degraded, and a re-test is recommended.
- In very rare cases, in spite of a live culture being submitted for testing, PCR tests may be equivocal, in which case a re-test should be done.

5. Where can I purchase culture pouches?

The most commonly used culture medium, the InPouch TF, is available from BioMed Diagnostics. BioMed also sells a *T. foetus* live culture (positive control); this is an excellent reference sample for clinics to compare to samples collected from client animals.

6. During incubation, some culture pouches blow up with gas and can't be read. How do I prevent this?

Prevent contamination during sample collection. Bacteria grow easily in the culture medium.

Ways to minimize bacterial overgrowth of samples:

- Use a new pipette for each collection
- Use pipettes covered in a soft plastic chemise; pop end of pipette through chemise only once it is in the proper location for sampling
- Trim preputial hair if long or dirty
- Flush prepuce with 60 – 120cc sterile, **non-bacteriostatic** saline if there is gross contamination such as mud or manure
- Re-sample if chunks of manure or debris are seen in culture pouch after inoculating